IN VITRO SEED GERMINATION AND CALLUS FORMATION OF DENDROBIUM MACROSTACHYUM

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ABSTRACT: The genus Dendrobium is one of the most important genera with distribution of eleven species in the Western Ghats, fit in to the family Orchidaceae. Dendrobium macrostachyum is an endemic and threatened species with pharmaceutical importance. In vitro studies will immensely aid conservation measures of this orchid species. MS, VW, B5 and KC media supplemented with various concentrations of auxins and cytokinins were used in combination for asymbiotic seed germination and plantlet formation. In the assessment of the media VW medium supplemented with BAP of 0.5 mg and 5 mg NAA was found to be most suitable for plantlet formation. VW basal medium fortified with Basal VW Media fortified with 1.5 mg BAP, 1.5 mg NAA, 50 ml CM and 500 mg AC was found to be most suitable for in vitro rooting. A hardened plant was transferred to green house after ex vitro rooting technique. Implication of the present work is discussed here.

KEY WORDS: Dendrobium macrostachyum,VW, MS, B5, KC, BAP, NAA, IAA,CM, AC, in vitro, callus formation, seed germination.

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ABBREVIATIONS

VW-Vacin and Went, MS- Murashige and Skoog, B5 - Gamborg B5 medium, KC – Knudsons Media, BAP – Benzyl Amino Protein, NAA–Naphthalene Acetic Acid, IAA–Indole Acetic Acid, CM–Coconut Milk, AC – Activated charcoal.

INTRODUCTION

The genus Dendrobium is the largest genus belonging to Orchidaceae, which is one of the largest flowering families. Most of the members are epiphytic with very few species being lithophytic[12]. Dendrobium macrostachyum [3, 4, 6] is an epiphytic orchid widely distributed in Kodagu (Anekadu) Mysore, Belgaum, Hassan, Shimoga, Kudremukh in Karnataka, Kerala, Maharashtra, Sri Lanka. It is pendant, has cord similar stem, leafless once flowering in fascicles from the nodes, with straw coloured petals and sepals. Lip has network of purple veins, inside. Flowering then Fruiting is between May to December. Spot character is that the lip is brownish to green in colour [13].

This species is an endemic and threatened species distributed in the South and South West parts of India[13]. D. macrostachyum population has been declining due to damage of habitat, unselective collections by the orchid enthusiasts [14]. Hence, a quick conservation method is an crucial need. In vitro studies drive to help in the conservation of this orchid species [1, 2, 7].

This plant is high in flavonoid content [10] and is of pharmaceutical importance and is used as a pain killer by tying plant materials overnight on the parts of body to relieve from pain. The

tender shoot tips are used as an ear drop for ear ache and also to treat boils, pimples and other skin eruptions. Secondary metabolism in this plant appears to be a resource of many biologically active metabolites [11].

In vitro asymbiotic seed germination on nutrient media[8] is a much faster and effective method for conservation [5] and mass multiplication of threatened and endangered orchids. Hence this investigation was undertaken for judicious use of growth regulators [9] during in vitro seed germination of Dendrobium macrostachyum.

Research Design:

Need and Importance of the Study – The study is done for learning and understanding the cultivation of orchids through tissue culture for genetic/multiplication of rare orchids - Dendrobium macrostachyum.

Dendrobium macrostachyum, is found in the western ghats more hogh, the study is observing to find the need of the cultivation and the importance from the need in decorative of house, etc.,

Research Methodology

Type of Research: Experimental Research

Type of Data: Primary Data – Experimental Analysis
Secondary Data – The data were published and unpublished materials.

Sampling Design: Judgmental Sampling

Data Collection: Seeds collected from Western Ghats – Shimoga

Limitations:

1. Tissue Culture – IVF
2. Natural Conditions take longer time, so practiced in tissue culture.
3. 1 month for plantlets, 3 months for complete Plants.

MATERIALS AND METHODS

Dendrobium macrostachyum specimens were collected from Sagar between 7th May 2012 and 9th May 2012. Sagar is located in the Western Ghats range and is known for its proximity to Jog Falls and to the historical places of Ikkeri and Keladi. Sagar is surrounded by water bodies and forest regions. Sagar is located at 14.17°N 75.03°E. The average weather conditions during visit were 25°C, Wind NE at 6 km/h and 58% Humidity.

Plant specimens were collected from the natural environment in perforated, clean, polythene bags. Care was taken to ensure to retain the mother plant intact in its natural epiphytic territory. Standard plant life were referred for validation of the genus and species. They were planted in

green house of St. Joseph's Post-Graduate and Research Centre. Herbaria were prepared using standard protocol and voucher specimens were deposited in the herbaria of St. Joseph's Post-Graduate and Research Centre, Bangalore.

1 – Habit of D. macrostachyum

2 – Collection of D. macrostachyum

Surface sterilization

Green capsules of wild were collected and then rinsed thoroughly three times with sterile distilled water, followed by dipping them in 70% ethanol aimed at 30 seconds. Sterilized capsules remained dried and then split longitudinally with sterile surgical blade. Seeds were inoculated on different nutrient media like MS medium, B_5 medium, KC medium and VW medium which were prepared with various concentrations and combinations of phytohormones and other additives. VW medium gave the best results in comparison to all supplementary media. So VW was consistent for Dendrobium macrostachyum.

Seed cultures were placed in growth chamber at 25 ± 20 °C and 70 –80% relative humidity under 24h-light and under 16h-light/8h-dark with light provided by cool white fluorescent lamps for 70 days. Sub-culturing was regularly done every 15 days and observations were made

In vitro rooting

In vitro rooting was successful with VW media supplemented with 1.5 mg BAP, 1.5 mg NAA, 50 ml CM and 500 mg AC.

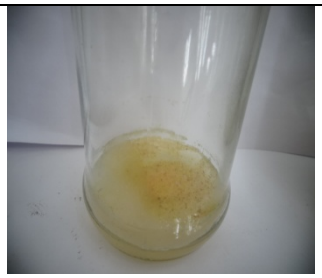
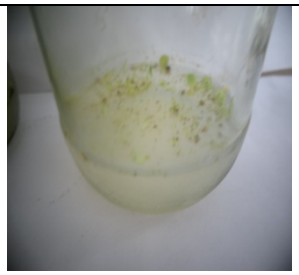
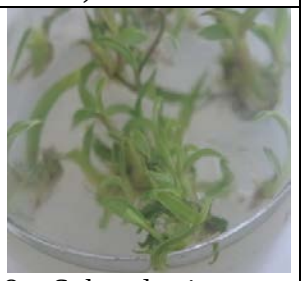
Ex vitro rooting

The basal ends of healthy shoots from the shoot multiplication medium were dipped in an auxin solution, 10 ml of IAA (made in distilled water) then planted in small pots containing solrite (potting mix) sprayed with bavistin to avoid fungal infection. In vitro rooted plants in the pot trays containing potting mixture maintained under mist chamber and covered with perforated plastic cups.

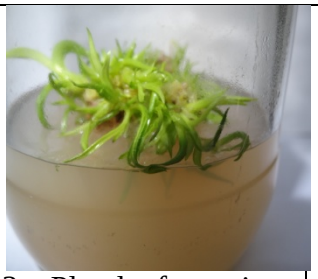
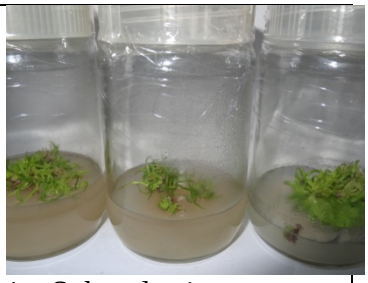
Hardening

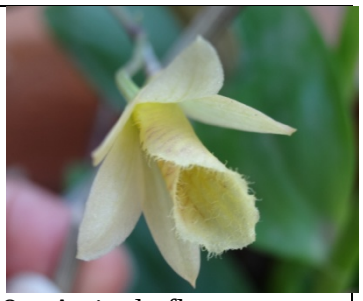
Well grown shoots were directly transferred to small pots containing soil, sand and solrite (mixture of pearlite and peat moss) and were kept in the green house. Effectively established plantlets were subsequently transferred to field condition.

OBSERVATIONS

 1 – Innoculation of seeds	 2 – Swelling of seeds (3 weeks)	 3 - Initial satges (4 weeks)	 4 - Initial stages (6 weeks)
 5 – Plantlet initiation	 6 – Plantlet formation	 7 - Plantlet formation	 8 – Sub-culturing
 9 – Invitro rooting	 10 – Ex vitro rooting	 11 – Hardening	 12 – Transfer to the greenhouse

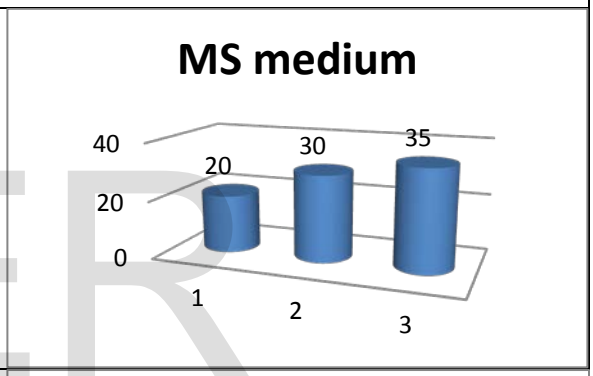
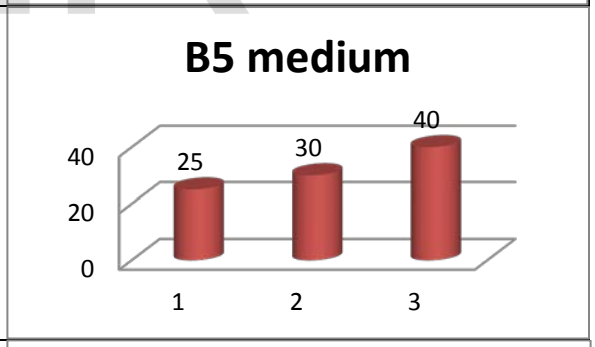
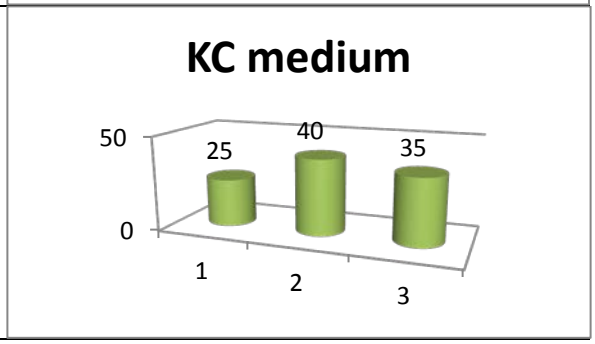
Callus formation :

 1 – Callus formation	 2 – Callus formation	 3 – Plantlet formation	 4 – Sub-culturing

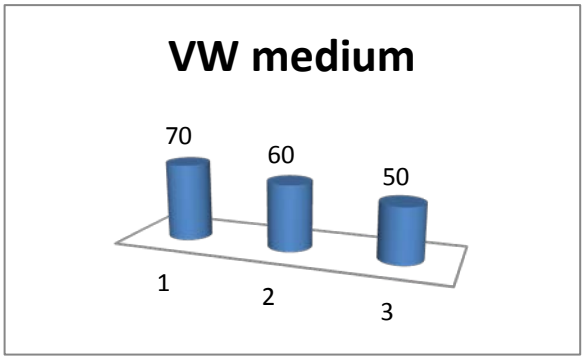
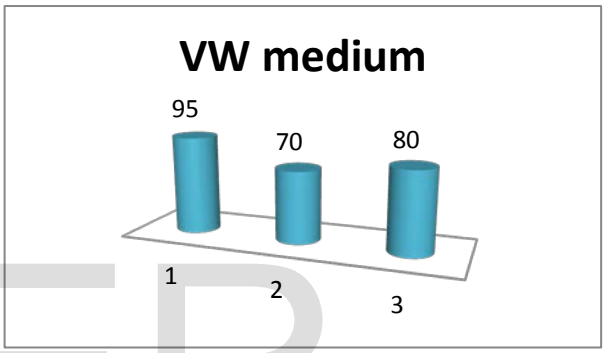
 5 - In vitro rooting	 6- Ex vitro rooting	 7 - Flowering in green house	 8 – A single flower

RESULTS AND DISCUSSION

MS, B5 and KC media was used (Table 1)

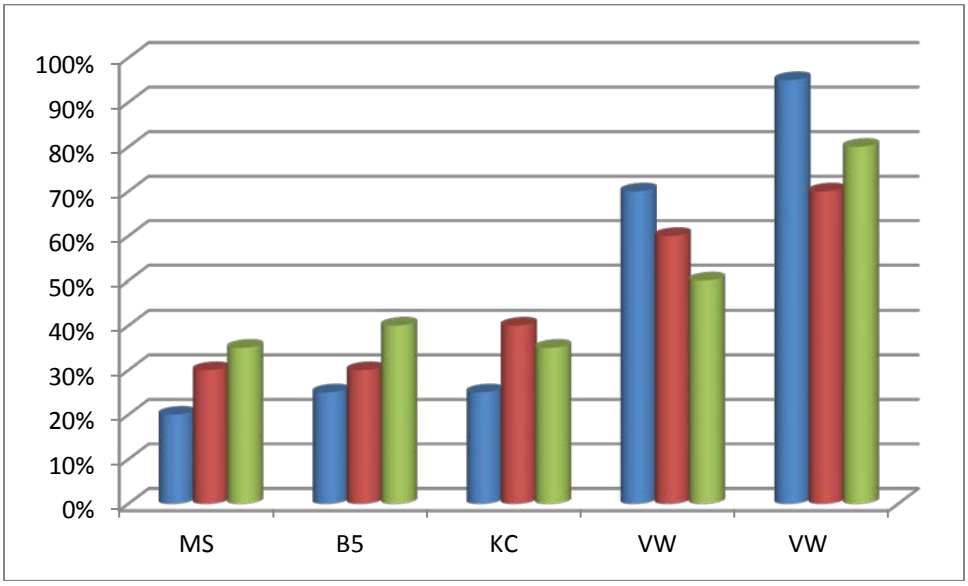
Media used	Media composition	Average plantlet formation (percentage)	
MS	Basal MS media + 1mg BAP + 1mg IAA Basal MS media + 2mg BAP + 1mg IAA Basal MS media + 3mg BAP + 1mg IAA	20% 30% 35%	
B_5	Basal B_5 media + 1mg BAP + 1mg IAA Basal B_5 media + 2mg BAP + 1mg IAA Basal B_5 media + 3mg BAP + 1mg IAA	25% 30% 40%	
KC	Basal KC media + 1mg BAP + 3mg IAA Basal KC media + 2mg BAP + 5 mg IAA Basal KC media + 3mg BAP + 10 mg IAA	25% 40% 35%	

Media Composition For Plantlet Formation (Table 2)

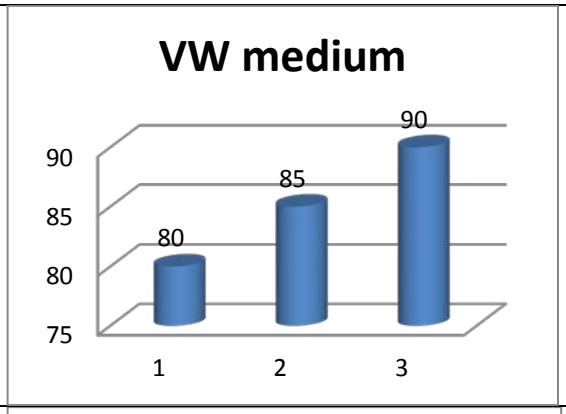
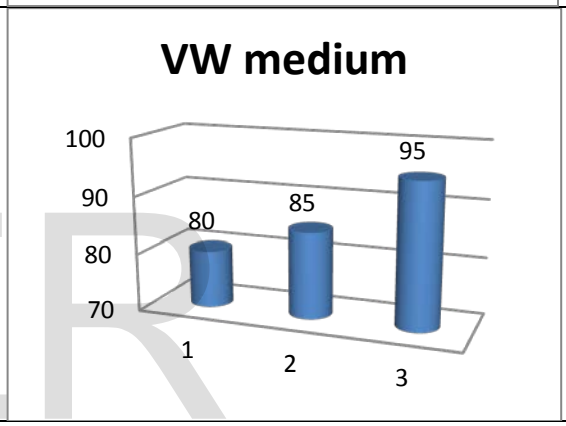
Media used	Media composition	Average plantlet formation (percentage)	
VW	Basal VW Media + 0.5 mg BAP + 0.5 mg NAA Basal VW Media + 1.0 mg BAP + 1 mg NA Basal VW Media + 2.0 mg BAP + 2 mg NAA	70% 60% 50%	
VW	**Basal VW Media + 0.5 mg BAP + 5 mg NAA** Basal VW Media + 1 mg BAP + 1 mg NAA Basal VW Media + 2 mg BAP + 2 mg NAA	**95%** 70% 80%	

In comparison to MS medium, B_5 medium and KC medium, VW medium gave best results and was standardized. Basal VW medium supplemented with 0.5 mg BAP and 5 mg NAA was found to be most suitable for plantlet formation.

Comparative plantlet formation based on different media used



Media Composition For Invitro Rooting (Table 3)

Media used	Media composition	Average plantlets showing rooting (%)	
VW	Basal VW Media + 1.5 mg BAP + 3 mg NAA + 500 ml CM + 200 AC Basal VW Media + 1 mg BAP + 4 mg NAA + 50 ml CM + 250 AC Basal VW Media + 0.5 mg BAP + 5 mg NAA + 50 ml CM + 500 mg AC	80% 85% 90%	
VW	Basal VW Media + 2.5 mg BAP + 2.5 mg NAA + 50 ml CM + 150 mg AC Basal VW Media + 2 mg BAP + 2 mg NAA + 50 ml CM + 250 mg AC **Basal VW Media + 1.5 mg BAP + 1.5 mg NAA + 50 ml CM + 500 mg AC**	80% 85% **95%**	

Germination was faster on the VW medium when compared to MS medium, KC and B5 media (Refer table 1). Attentiveness of VW medium supplemented with 0.5 mg BAP and 5 mg NAA was found to be most suitable for plantlet formation (Refer table 2). VW medium supplemented with 1.5 mg BAP, 1.5 mg NAA, 50 ml CM and 500 mg AC was found to be suitable for In vitro Rooting (Refer table 3). Ex-vitro rooting was done by spraying with IAA and potting in solorite. The plants with good rooting were transferred to community pots and then to greenhouse conditions.

CONCLUSION

From these studies it can be concluded that the VW medium is most suitable for Dendrobium macrostachyum seed germination. This study also revealed that a low concentration of 0.5 mg BAP and 5 mg NAA was found to be more suitable for plantlets and multiple plantlets**.** VW medium supplemented with 1.5 mg BAP, 1.5 mg NAA, 50 ml CM and 500 mg AC was found to be suitable for In vitro Rooting.

SCOPE

1. In vitro micropropagated plants can be shifted to natural habitats of Western ghats to facilitate In situ conservation of Dendrobium macrostachyum.

2. Using elicitors (From biological origin) for enhanced plantlet formations.
3. Several species of Dendrobium have also been used in Chinese medicine (Hu, 1970) and in Indian system of medicine (Misra, 2007) to cure cough, cold etc. This plant is of pharmaceutical importance and is used as a painkiller by tying plant materials overnight on the parts of body to discharge from pain. The caring shoot tips are used as an ear drop for earache and also to extravagance boils, pimples and other skin eruptions. Secondary metabolism in this plant appears to be a resource of many biologically active metabolites. (Nimisha.P.S and Hiranmai Yadav.R. 2012 Proximate And Physicochemical Analysis Of Dendrobium Macrostachyum Lindl, International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491 Vol 4, Issue 1, 2012)

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